VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at page 10, line 16 has been amended as follows:

--The present invention provides a method for detecting water-borne microorganisms which serve as an indicator of the probable presence of pathogens, primarily of fecal origin. In a preferred embodiment, the method comprises the following steps: (1) providing a liquid or liquified sample (which may include treating the sample with a culture media to enrich microorganisms present in the sample); (2) recovering microorganisms from the treated sample; (3) lysing the recovered microorganisms to release substantially undegraded DNA therefrom; (4) providing specific primers which will hybridize to separated target strands of a target nucleotide sequence from the target gene of the microorganism of interest and amplify the target nucleotide sequence but not sequences found in other organisms; (5) mixing the primers with the recovered DNA; (6) amplifying the specific target nucleotide sequence with polymerase thereby extending the primers to make fully double-stranded replicas of the target DNA sequence; (7) detecting the presence of the amplicon (amplified target DNA sequence) by binding the amplicon to a microtiter plate and staining with a stain such as **[PicoGreen]** **PICOGREEN**, which selectively stains double stranded DNA; and (8) making a determination about the presence or absence

of the microorganism in the original liquid or liquified sample based on presence or absence of the amplicon. Examples of the primers which may be used, and which are discussed in more detail below, are shown in Tables I, and II, III and IV.--

Paragraph beginning at page 32, line 19 has been amended as follows:

--In a preferred version, the detection reagent is the substrate **[PicoGreen]** **PICOGREEN**. The use of **[PicoGreen]** **PICOGREEN** as a stain for double stranded DNA (dsDNA) is described in U.S. Pat. No. 5,824,557 entitled, "Method for Detecting and Quantitating Nucleic Acid Impurities in Biochemical Preparations", issued Oct. 20, 1998 to T. J. Burke *et al* and assigned to PanVera Corporation, which is hereby expressly incorporated herein by reference. **[PicoGreen]** **PICOGREEN** is an ultra sensitive fluorescent nucleic acid stain which is ideal for quantitating dsDNA in the microtiter plate format. The ability of this reagent to detect very small amounts of dsDNA in the presence of contaminating RNA, single stranded DNA (ssDNA) or proteins, combined with its wide linear response, provides significant advantages over other methods.--

Paragraph beginning at page 33, line 8 has been amended as follows:

--Free **[PicoGreen]** **PICOGREEN** dye is essentially nonfluorescent

and exhibits a greater than 1,000-fold fluorescence enhancement upon binding to dsDNA. **[PicoGreen]** **PICOGREEN** staining is highly selective for dsDNA over RNA, ssDNA and oligonucleotides, and is not compromised by the presence of proteins, nucleotides and other sample contaminants in the reaction.--

Paragraph beginning at page 33, line 14 has been amended as follows:

--However, it will be understood by a person of ordinary skill in the art that although **[PicoGreen]** **PICOGREEN** is a preferred stain, other detection reagents, including other dsDNA stains well known to persons of ordinary skill in the art, may be used in the present invention.--

Paragraph beginning at page 33, line 19 has been amended as follows:

--The detection reagent controls which contain only **[PicoGreen]** **PICOGREEN** (or other detection reagent) and each of the primer sets are used to determine a baseline fluorescence for determining presence or absence of *E. coli* and/or *Enterococcus faecalis/faecium*.--

Paragraph beginning at page 34, line 16 has been amended as follows:

--The detection method as described in detail herein is designed to detect the presence or absence of the indicator organism. However, a semiquantitative result can also be obtained using a similar method. In this method, to a series of wells is added increasing known amounts of *E. coli* or *Enterococcus* so that a standard curve can be constructed. The result obtained from the DNA sample collected from a liquid or liquified sample can be directly compared to the standard curve to determine the approximate amount of indicator organism present in the liquid or liquified sample. The use of **[PicoGreen] PICOGREEN** or other detection reagents will allow for quantitation of the dsDNA present, and the assay will display a linear correlation between dsDNA concentration and fluorescence, allowing a detection range extending from about 25 pg/ml to about 1 µg/ml dsDNA using a single **[PicoGreen] PICOGREEN** dye concentration.--

Paragraph beginning at page 35, line 12 has been amended as follows:

--In one embodiment, a desired kit for use in a method for detecting *Escherichia coli* in a liquid or liquified sample comprises a primer set for amplification of a sequence in the *lamB* gene. The primer set is selected from the primer sets described in Table V or elsewhere herein such as primer sequences of up to 40 bp comprising SEQ ID NO:1 and SEQ ID NO:14. One of the two primer

sequences of the primer set provided in the kit will be biotinylated. The kit may also include a detection reagent, such as **[PicoGreen]** **PICOGREEN**, for detection of an amplified sequence in the *lamB* gene, and a detection well having streptavidin coated thereon wherein the amplified sequence is detected by the detection reagent.--

Paragraph beginning at page 36, line 1 has been amended as follows:

--In another embodiment, a desired kit for use in a method for detecting *Enterococcus faecalis* and/or *Enterococcus faecium* in a liquid or liquified sample comprises a primer set for amplification of a sequence in the transposase gene Tn1546. The primer set is selected from the primer sets described in Table VI or elsewhere herein such as primer sequences of up to 40 bp comprising SEQ ID NO:27 and SEQ ID NO:32. One of the two primer sequences provided in the kit will be biotinylated. The kit may also include a detection reagent, such as **[PicoGreen]** **PICOGREEN**, for detection of an amplified sequence in the transposase gene Tn1546, and a detection well having streptavidin coated thereon wherein the amplified sequence is detected by the detection reagent. As noted above, kits may comprise primer sets for both *E. coli* and *Enterococcus faecalis/faecium* and may further comprise more than one primer set appropriate for each species of bacteria.--

In the Claims:

Claim 7 and 15 have been cancelled.

Claims 1-3, 6, 8, 13-14, and 16-18 have been amended as follows:

1. (Twice Amended) An oligonucleotide primer having up to 40 bases and comprising the sequence SEQ ID NO:1; **[SEQ ID NO: 2; SEQ ID NO:3;]** **or** SEQ ID NO:14**[; SEQ ID NO:15; or SEQ ID NO:16]** **wherein the primer is specific for the detection of E. coli**.

2. (Once Amended) **[The]** **An** oligonucleotide primer **[of claim 1]** having 23-40 bases and comprising **[SEQ ID NO:1;]** SEQ ID NO:2; or SEQ ID NO:3 **wherein the primer is specific for the detection of E. coli**.

3. (Once Amended) **[The]** **An** oligonucleotide primer **[of claim 1]** having 23-40 bases and comprising **[SEQ ID NO:14;]** SEQ ID NO:15; or SEQ ID NO:16 **wherein the primer is specific for the detection of E. coli**.

6. (Once Amended) A method of **specifically** detecting **[microorganisms]** **E. coli** in a liquid or liquified sample by polymerase chain reaction, comprising:

providing a liquid or liquified sample;

recovering bacteria from the liquid or liquified sample;

lysing the bacteria to provide a DNA sample; **[and]**

treating the DNA sample under PCR conditions with a primer set **specific for E. coli** for **[detecting the presence of]** **forming an** amplified DNA **[as an indication of the presence of *Escherichia coli* in the liquid or liquified sample]** wherein the primer set comprises SEQ ID NO:1 and SEQ ID NO:14**[; SEQ ID NO:2 and SEQ ID NO:15; or SEQ ID NO:3 and SEQ ID NO:16.]** **; and**

detecting the presence of said amplified DNA as an indication of the presence of E. coli in the liquid or liquified sample.

8. (Once Amended) The method of claim 6 wherein in the step of detecting the presence of amplified DNA, **the** presence of *Escherichia coli* is indicated when a signal is obtained which exceeds a predetermined threshold.

13. (Twice Amended) A method of **specifically** detecting **[bacteria]** **E. coli** in a liquid or liquified sample by polymerase chain reaction, comprising:

providing a liquid or liquified sample;

recovering bacteria from the liquid or liquified sample;

lysing the bacteria to provide a DNA sample;

selecting a target gene **of E. coli** and selecting **[a] an E. coli-specific** target DNA sequence in the target gene;

incubating the DNA sample under amplification conditions with a DNA polymerase and a primer pair **specific for E. coli** for amplifying the target DNA sequence; and

detecting the presence of amplified DNA as **[an] a specific** indication of the presence of **[bacteria] E. coli** carrying the selected **E. coli-specific** target DNA sequence, wherein the target gene is the *lamB* gene of *Escherichia coli*.

14. (Once Amended) A kit for use in **specifically** detecting *Escherichia coli* in a liquid or liquified sample, the kit comprising a primer pair having a first primer comprising **[an oligonucleotide primer of claim 2,] SEQ ID NO: 1** and a second primer comprising **[a corresponding oligonucleotide primer of claim 3.] SEQ ID NO: 14.**

16. (Once Amended) The kit of claim 14 further comprising a detection agent for detection of **[the]** amplified DNA **produced using the primer pair under amplification conditions**.

17. (Once Amended) The kit of claim 16 wherein the detection reagent is **[PicoGreen]** **a dsDNA stain**.

18. (Once Amended) The kit of claim **[14]** **16** further comprising a detection well having streptavidin coated thereon wherein the amplified DNA sequence is detected by the detection reagent.